Seroprevalence of bovine viral diarrhea virus neutralizing antibodies in finisher hogs in Ontario swine herds and targeted diagnostic testing of 2 suspect herds

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Abstract — A pilot study was initiated to determine the seroprevalence of bovine viral diarrhea virus (BVDV) neutralizing antibodies in finisher hogs in Ontario swine herds, including 2 swine herds with clinical syndromes suspicious of BVDV. No herds were positive for BVDV antibodies by virus neutralization. The 2 swine herds with clinical disease suggestive of pestivirus infection were also negative for antibodies to BVDV in indirect fluorescent antibody assays. Prevalence of BVDV in Ontario swine farms is negligible.

Résumé — Séroprévalence des anticorps neutralisants du virus de la diarrhée virale des bovins chez des porcs en finition de troupeaux porcins de l'Ontario et tests de diagnostic dans 2 troupeaux suspects ciblés. Une étude pilote a été entamée pour déterminer la séroprévalence des anticorps neutralisants du virus de la diarrhée virale des bovins (BVDV) chez des porcs en finition de troupeaux porcins de l'Ontario, incluant 2 troupeaux porcins avec des syndromes cliniques suspects du BVDV. Aucun troupeau n'a été trouvé positif pour les anticorps du BVDV par la neutralisation du virus. Les 2 troupeaux porcins avec une maladie clinique suggérant l'infection par le pestivirus ont aussi eu des résultats négatifs pour les anticorps au BVDV dans les tests d'immunofluorescence indirecte pour les anticorps. La prévalence du BVDV dans les élevages porcins de l'Ontario est négligeable.

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Pestiviruses are important pathogens of cattle, swine, and sheep. Classified as a genus within the family *Flaviviridae,* pestiviruses are small, enveloped, predominately non-cytopathogenic, positive-stranded RNA viruses (1). The genus *Pestivirus* contains 4 species currently recognized by the International Committee on Taxonomy of Viruses, namely, classical swine fever virus (CSFV or hog cholera virus), bovine viral diarrhea virus type 1 (BVDV-1) and type 2 (BVDV-2), and border disease virus (BDV) of sheep (2,3). Bovine viral diarrhea virus under natural conditions mainly infects cattle and sheep; however, the virus has been associated with disease in goats and swine as well (1). In swine, infection with BVDV is often subclinical but when clinical signs are reported they are often associated with reproductive problems such as poor conception rates, abortion, and stillborn piglets (1,4).

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Interest in the prevalence and clinical significance of pestivirus in swine herds has recently been highlighted with the documentation of a neurologic and reproductive disease outbreak associated with a novel pestivirus in the USA (5) and a disease outbreak in Australia (6). Pogranichniy et al (5) reported neurological signs in weaned pigs consisting of posterior weakness, paresis, ataxia, lameness, and head pressing. Sows in this study showed similar neurological signs as well as reproductive problems including early return to estrus or abortion after 30 d of gestation (5). Histopathological evaluation of the affected sows detected encephalitis characterized by mild focal non-suppurative perivascular cuffing and gliosis (5). Kirkland et al (6) reported a slightly different clinical picture associated with a pestivirus outbreak but the clinical signs encompassed both neurological and reproductive abnormalities. These authors reported sudden death in piglets 3 to 4 wk of age and a marked increase in the birth of stillborn and mummified fetuses (6). Histopathological changes identified in this outbreak consisted primarily of multifocal non-suppurative myocarditis (6). A polymerase chain reaction (PCR)-based screening test was used to follow the cases reported in the United States and it was determined that the pestivirus isolated in this instance was not the same pestivirus that had been identified in Australia (7).

Herd prevalence levels of BVDV on North American swine herds is reported to be anywhere from 2% to 43% with cattle being implicated as the most common source of BVDV in pigs (1,4). Novel strains of pestivirus have yet to be identified in Ontario swine herds and the current prevalence of BVDV in

Ontario swine herds is unknown. Swine herds with neurological and reproductive syndromes of unknown etiology, referred to the swine field services veterinarians at the Ontario Veterinary College (OVC), University of Guelph, raised suspicions that these syndromes could be associated with BVDV infection.

The objectives of this study were to determine the prevalence of neutralizing antibodies to BVDV type-1 and BVDV type-2 in Ontario swine finishing pigs and to investigate herds referred to the swine veterinarians at the OVC for the presence of BVDV neutralizing antibodies.

To determine the prevalence of BVDV neutralizing antibodies in Ontario swine herds, 500 samples of stored finisher pig sera from swine sentinel herds were analyzed. The stored samples were from 50 swine herds that participated in a swine sentinel herd program at the University of Guelph in 2005 (8). The Ontario sentinel herds were distributed across the province and herd level information and blood samples from each herd were collected for this program. For the pilot study, a systematic random sampling method was employed by selecting every other serum sample resulting in the selection of 10 serum samples from each of the 50 sentinel herds. The degree of contact with cattle for each herd was compiled from the sentinel project herd records (8).

The serum samples were tested by virus neutralization (VN) for BVDV-type 1 and BVDV-type 2 by the Animal Health Laboratory (AHL) at the University of Guelph, using a conventional microtiter virus neutralization assay performed in cell culture as described by Carman et al (9). Two-fold serial dilutions of serum were prepared in duplicate starting at 1:2 and incubated with either 100 cell culture infective doses (CCID) of BVDV type 1-NADL or BVDV type 2-NVSL-125c for 1 h at 37°C, followed by addition of Madin-Darby bovine kidney (MDBK) cells suspended in growth medium supplemented with fetal equine serum. After incubation at 37°C for 3 d, each well was analyzed for cytopathic effects within the cell monolayer. The titer was determined as the 50% endpoint where half of the cells were protected. Known positive and negative serum controls were included for each assay.

Two problem herds referred to the OVC swine field service department were experiencing neurologic and reproductive problems similar to the report of Pogranichniy et al (5). These herds were selected for targeted testing for BVDV. The first herd was a 250 sow farrow-to-finish operation in southwestern Ontario experiencing an outbreak of reproductive losses of undetermined etiology. Clinical signs included mid- to late-term abortions and an increase in numbers of sows returning to estrus. The outbreak occurred during June–July 2008. Affected sows ranged from parity 1–5. Infection with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type-2 (PCV-2) was previously ruled out by the referring veterinarian. Blood samples were taken from 12 of the affected sows, after sufficient time had elapsed to allow for the development of antibodies (> 30 d), and were submitted to the AHL for BVDV VN testing as described herein for the sentinel herds. In addition, these 12 samples were tested for IgG antibody to BVDV using an indirect fluorescent antibody test (IFA) (10), with BVDV type 1-NADL and BVDV type 2-NVSL-125c infected and uninfected secondary bovine spleen cell cultures grown in 8-chamber slides and fixed in cold acetone. The swine sera were tested at a 1/20 dilution. Phosphate-buffered saline, pH 7.2, was used as the washing buffer. The secondary antibody, anti-swine IgG conjugated with fluorescein, was used at a dilution of 1/50. The stained slides were covered with mounting fluid at pH 9.0, mounted with a cover glass, and examined under a fluorescence microscope. Known bovine BVDV IgG positive and negative sera were used as controls.

The second herd was a 70-sow farrow-to-finish operation in southwestern Ontario experiencing an outbreak of neurologic signs in weaned pigs approximately 5 to 6 wk of age. The outbreak occurred in November 2008. Apparently healthy weaned pigs developed sudden onset of CNS dysfunction characterized by complete body tremors, ataxia, head pressing, and death. An affected animal was submitted to the AHL for euthanasia, gross postmortem examination, histopathology, immunohistochemisty (IHC) for PCV-2, and reverse transcription–polymerase chain reaction (RT-PCR) for PRRSV. PRRSV testing was done using the real-time PRRSV North American/European-RT-PCR kit, manufactured by Tetracore (Rockville, Maryland, USA), with modifications to adapt to an ABI-7500 thermocycler (11). The test uses primer sets directed to the 3′ untranslated region of the PRRSV genome. Immunohistochemistry for PCV-2 antigen was performed as described by Carman et al (12). In addition to the animals submitted for histopathology, 20 serum samples were collected from piglets in the same batch as the affected animals showing neurological symptoms, after sufficient time had elapsed for the development of antibodies (> 30 d). The serum samples were tested for antibody to BVDV-1, and BVDV-2 by virus neutralization as described for the sentinel herds.

All 500 samples from the 50 sentinel herds tested negative (titers < 1:2) for antibody to BVDV-type 1 and BVDV-type 2. Of the 47 herds with information available on the presence of cattle, cattle were present on 27.6% of the farms. On the remaining 72.3% of the farms, only pigs were present.

All 12 affected sows from herd #1 were negative for virus neutralizing antibodies for BVDV, and negative by IFA test at a 1:20 dilution. The herd was negative for PRRSV and PCV-2 as reported by the referring veterinarian. Histopathology results for the specimen submitted from herd #2 demonstrated a mild non-suppurative encephalomyelitis and Purkinje cell degeneration and heterotopia suggestive of an *in utero* virus infection causing cerebellar dysplasia and atrophy. RT-PCR for PPRSV was negative. IHC PCV-2 staining was not apparent in any of the inflammatory lesions. The 20 serum samples from herd #2 were also negative for BVDV neutralizing antibodies.

The results indicate that BVDV did not appear to be present in Ontario swine herds in 2005 regardless of the presence of cattle on the same premise. These findings are consistent with other reports in which the seroprevalence of ruminant pestiviruses in swine was found to be low (13). The presence of cattle on the same premise did not appear to be a risk factor in this study. However, our sample size was low for herds with cattle on the same premise, and without a positive case in either group, the risk that cattle contribute to the prevalence of seropositivity to BVDV could not be assessed. The BVDV status of the

cattle associated with the swine sentinel herds and case herd #2 was not available. However, the herd prevalence of BVDV on Ontario dairy farms has been estimated at 29% and 13% for BVDV-type 1 and BVDV-type 2, respectively (14). The neurologic and reproductive problems in herds #1 and #2 could not have been related to BVDV. Herd #2 had cattle present on the same farm, while herd #1 had exclusively swine.

The Ontario swine industry is experiencing a rapid decline in the presence of mixed farming practices and has moved almost exclusively to species specialization on farms, a phenomenon witnessed in many parts of the world today (15). It has been speculated that this trend in farming practices has resulted in the decline of BVDV in swine worldwide (1,13). However, despite the perceived decline of BVDV in swine herds there have been recent reports of novel pestiviruses within the species (5,6). Fortunately, such novel forms of pestivirus have yet to be reported in Ontario swine herds.

While BVDV was not identified in this study, the investigation and identification of novel pestiviruses, and the continual monitoring and understanding of existing pestiviruses should remain an area of active interest for animal health researchers, virologists and animal disease surveillance networks. The significant role that pestiviruses have played in animal disease outbreaks, international trade barrier restrictions, and their demonstrated potential for cross species spread has generated a continued need for the investigation of existing pestiviruses and new species of pestivirus as they emerge.

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